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(71) Applicant *(for all designated States except US)*: APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. [NL/NL]; 6 John B. Gorsiraweg, Curaçao (AN).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : SAMARITANI, Fabrizio [IT/IT]; Via Luigi Chiala, 130, I-00139 Rome (IT). NATALE, Patrizia [IT/IT]; Via Tirso, 80, I-00198 Rome (IT).

(74) Agent: VANNINI, Mario; Istituto Framacologico Serono SpA, Via Gregoriana, 38 - 4th Floor, I-00187 Rome (IT).

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(54) Title: GONADOTROPIN CONTAINING PHARMACEUTICAL COMPOSITIONS WITH SUCROSE STABILIZER

(57) Abstract

Pharmaceutical compositions containing FSH, LH or hCG stabilized by means of sucrose. The formulation is particularly suitable for stabilizing a lyophilisate of recombinant gonadotropins.

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GONADOTROPIN CONTAINING PHARMACEUTICAL COMPOSITIONS WITH SUCROSE STABILIZER

The present invention concerns gonadotropin containing pharmaceutical compositions. More precisely, it concerns compositions of sucrose-stabilized gonadotropins. The gonadotropins of the present invention comprise FSH (Follicle Stimulating Hormone), LH (Luteinizing Hormone) and hCG (Human Chorionic Gonadotropin).

It is known that highly purified proteins are time-unstable and are stabilized, for instance, in admixture with saccharides, such as lactose and mannitol, or else with proteins and aminoacids, such as albumin and glycin. Other high-molecular-weight compounds, having a biological origin, as, for instance, the marine colloids, dextran and other polysaccharides and the phospholipids often work equally well. Anyway, since the gonadotropins of the present invention are administered parenterally, these excipients are not suited for an injectable composition because of their allergenicity or their insufficient solubility, in some cases because of their potential toxicity or a concourse of these effects.

The composition of lyophilised proteins is described in M.J. Pikal, BioPharm, October 1990, 25-30. There are reported examples of proteins, such as growth hormone and ribonuclease A, formulated by using stabilizing excipients such as mannitol, glycin, arginine and lactose.

In particular, the lyophilisation is described of proteins in the presence of various substances in their amorphous state, as sugars, which increase the

collapse temperature and permit to obtain shorter lyophilisation times. However, it is not feasible, according to the author, to foresee a standard formulation for all the proteins, and the choice of the best formulation requires a remarkable selection work.

German patent DE 3520228 describes bioactive proteins such as lymphokines, interferons, TNF (Tumor Necrosis Factor), insulin, growth hormone, in formulations which are stabilized by means of polysaccharides comprising repetitive maltotriose units. The use of sucrose as a stabilizing agent is known, for instance, in a formulation of lyophilized orgotein, as described in US patent 3,637,640. International patent application WO 89/10407 describes the formulation with sucrose of M-CSF (Macrophage-Colony Stimulating Factor); patent application WO 89/09610 describes, instead, formulations of TNF which have been stabilized with albumin, dextran, polyethylene glycol, 80 polysorbate PVP, lactose, trialose or even sucrose.

The injectable formulations of gonadotropins are obtained by a process which includes their lyophilisation in order to obtain a dry powder. Gonadotropins are highly liable to denaturization during the lyophilisation process and it is desirable to obtain stable formulations able to maintain a longer cycle life when they are stored at room temperature.

European Patent EP 448146 describes lyophilised gonadotropin containing preparations, which are stabilized by means of a bicarboxylic acid salt, as, for instance, citric acid, tartaric acid and aspartic acid. Gonadotropins which are found on the market are stabilized by means of saccharides, for instance hCG is stabilized by means of mannitol (Profasi^{R}, SERONO) and

FSH is stabilized by means of lactose (Metrodin^R, SERONO).

We have now found that sucrose confers a better stability to the formulation of gonadotropins and in particular to the form of these glycoproteins which have been prepared with the recombinant DNA technique.

The main object of the present invention is to provide a pharmaceutical composition comprising a solid intimate mixture of a gonadotropin, such as FSH, LH or hCG, and a stabilizing amount of sucrose, alone or in combination with other stabilizing agents.

A further object is to provide a process for the preparation of said pharmaceutical composition, the step of lyophilising an aqueous solution of the components. Another object is to provide a presentation's form of said pharmaceutical composition comprising the said solid mixture hermetically closed in a sterile condition within a container suitable for storage before use and suitable for reconstitution of the mixture for injectable substances.

An other object is to provide a solution for said solid mixture reconstituted into an injectable solution. In order to evaluate the excipient's effect on the stability of the active ingredients, various formulations of recombinant FSH containing 150 I.U. pro vial have been prepared with various excipients: lactose, sucrose, glycin, sucrose plus glycin, lactose plus albumin and lactose plus glycin. All the formulations have been prepared by dissolving the excipients in phosphate buffer at pH 7, except the formulation with lactose (10 mg) which has been dissolved into H_2O for injection and adjusted at pH 6.4.

The samples have been stored at 45°C and tested

with a biological assay at fixed intervals of time. Tables 1 and 2 give the results of the tests effected on two different batches of recombinant FSH in the presence of different excipients, after 2 and 4 weeks for batch 1 (Tab.1) and after 1 and 3 weeks for batch 2 (Tab. 2).

The biological tests have been performed in compliance with the regulations of the European Pharmacopoeia and effected in duplicate. The tests for FSH and LH are reported in the "Menotropin" monography, whereas the test for hCG is reported in the "Chorionic Gonadotropin" monography.

The results show that the most stable formulations among those tested are those containing sucrose, i.e. formulations with sucrose alone and with sucrose plus glycin. Sucrose shows, surprisingly, to be an efficient stabilizing agent against the denaturization of the gonadotropins.

The stabilizing agents which are employed in the compositions of the present invention include, therefore, sucrose alone or in combination with other excipients, preferably aminoacids such as glycin. In particular, the stability has been studied of recombinant FSH and recombinant LH.

The gonadotropins produced according to the technique of recombinant DNA must be subjected to a high purification process in order to avoid contamination agents having a non-human origin and this high purity renders them less stable than the corresponding urinary gonadotropins.

The recombinant gonadotropins of the present invention have been prepared by expression in CHO (Chinese Hamster Ovary) cells, transformed with the corresponding recombinant DNA, according to the

Tab. 1

Batch 1 of recombinant FSH I.U.

Excipient	Amount (mg)	Theoretical titer	T=0	45° C 2 weeks	45° C 4 weeks
Lactose	10	167.31	129.0	139.0	104.0
Lactose	30	167.31	132.0	118.0	116.0
Sucrose	30	167.31	158.0	163.0	136.0
Sucrose	50	167.31	140.0	135.0	150.0*
Sucrose + Glycin	20 +10	167.31	144.0	143.0	186.0
Lactose + Albumin	20 + 3	167.31	127.0*	134.0	128.0
Glycin	20	167.31	132.0	107.0	-
Lactose + Glycin	20 +10	167.31	153.0	132.0	104.0

* valid only one assay

Tab. 2

Batch 2 of recombinant FSH 150 I.U.

Excipient	Amount (mg)	Theoretical titer	T=0	45° C 1 week	45° C 3 weeks
Lactose	10	155.08	163.0	121.0	103.0
Lactose	30	155.08	164.0	166.0	108.0
Sucrose	30	155.08	165.0	128.0	151.0
Sucrose	50	155.08	150.0	143.0	157.0
Sucrose + Glycin	20 + 10	155.08	160.0	152.0	185.0
Lactose + Glycin	20 + 3	155.08	172.0	136.0	101.0
Glycin	20	155.08	136.0	115.0*	97.0*
Lactose + Albunin	20 + 10	155.08	171.0*	137.0	157.0

* valid only one assay

technique described in European patents EP 160699 and EP 211894.

The close study of recombinant-FSH-containing formulations has been performed by using different compositions, according to the lay-out of Tab.3, respectively comprising: a) lactose, b) sucrose, c) sucrose plus glycin.

The preparation of the lyophilisate has been performed by diluting the bulk of gonadotropin with a solution of the excipient in water for injection ("a" formulation) or 0.01 M phosphate buffer ("b" and "c" formulations) in order to achieve the concentration of 200 I.U./ml, adjusting the pH at 6.4 for the lactose-containing formulations and at 7 for the sucrose containing or sucrose-plus-glycin-containing formulations. The solution has been filtered, brought to the final volume with the remaining solution of the excipient in order to achieve the concentration of 150 I.U./ml and lyophilized.

The accelerated stability of these formulations has been studied so that the stability of the same can be foreseen when they are stored in containers at room temperature, through the extrapolation of the data obtained at higher temperatures (+37°C; +45°C; +50°C).

The accelerated stability of the FSH formulations has been determined through the biological activity test, performed at the time intervals which are reported in the corresponding Tables.

Two ampoule preparations of HMG (Menotropin) have been used as standard solutions, the first having a biopotency of 101.3 I.U. FSH/ampoule and 85.6 I.U. LH/ampoule, the second having a biopotency of 103.1 I.U. FSH/ampoule and 82.3 I.U. LH/ampoule. The samples, at

Tab. 3

Formulations of recombinant FSH

Composition	Dosimetry U.I.	Lactose mg	Sucrose mg	Glycin mg	$Na_2HPO_4.2H_2O$ mg	$NaH_2PO_4.H_2O$ mg
a	150	10	-	-	-	-
b	150	-	30	-	1.11	0.45
c	150	-	20	10	1.11	0.45

the concentrations 0.5; 1.0 and 2.0 I.U./ml, as well as the standard HMG solutions, have been administered to three different groups of five rats each, through subcutaneous injection of 0.5 ml/rat twice a day for three consecutive days (final doses: 1.5; 3.0; 6.0 I.U. FSH/rat). Each animal has further received altogether a dose of 40 I.U. hCG.

Data reported in Tab. 4 refer to formulations of 3 different batches of recombinant FSH, containing 150 I.U. /ml FSH, in the presence of 10 mg lactose (Composition a), 30 mg sucrose (Composition b) and 20 mg sucrose plus 10 mg glycin (Composition c) in 5 ml vials. The tests have been performed at the temperatures 37°C, 45C° and 50°C.

The degradation is significant for the lactose containing formulations for all the test temperatures and for all the three batches. On the contrary, no appreciable variation is observed for the sucrose containing formulation of batch 1 at the same temperatures. For the formulation containing sucrose plus glycin relating to the first batch, the only appreciable degradation is observed at 50°C. For the formulations with sucrose or sucrose + glycin of the remaining batches, a degradation is observed which is lower anyway than that of the lactose containing formulation.

Tab. 5 gives further accelerated stability date, derived by the biological activity data, for 2 different batches (batch 1 and batch 2) of recombinant FSH formulations containing 150 I.U./ml FSH and 30 mg sucrose in 3 ml vials.

The study has been performed on vials stored for 5 weeks at the temperature of 50°C or for 10 weeks at the

Tab. 4

Study of the accelerated stability of recombinant FSH formulation (3 different batches - Batch 1, Batch 2 and Batch 3 containing 150 I.U./ml FSH) with lactose 10 mg (Composition a), sucrose 30 mg (Composition b) and sucrose (20 mg) + glycin (10 mg) (Composition c) in 5 ml vials.

Batch 1

		50°C			45°C					37°C			
	T=0	1W	3W	5W	2W	4W	8W	10W	11W	3W	5W	9W	12W
a	147	126	124*	83	163*	84	105	109	-	140*	109	97	122*
b	156	154	155	151	154	119	115	-	164	165	165	168	151
c	160	179	143	114	160	134	133	148	-	136	156*	132	175

Tab. 4 (Cont.)

Batch 2

		50°C			45°C				37°C			
	T=0	1W	2W	5W	2W	6W	8W	10W	5W	7W	10W	12W
a	135	50*	40*	-	96*	51	43	-	134	108	104	90
b	112	152*	134	96	125	94	89	101	157	163	135	114*
c	145	173*	124	118	143	145	154	135	146	154	139	141

Batch 3

		50°C			45°C				37°C			
	T=0	1W	3W	5W	2W	4W	8W	10W	3W	5W	9W	12W
a	144	40*	30*	-	135	30*	20*	-	106	70	34	-
b	152	136	138	110	161	136	106	110	165	179	159	140
c	135	140	176	125	163	142	122	125	151	151	158	176

W = weeks

* = only one assay valid

Tab. 5

Study of the accelerated Stability of recombinant FSH formulations (105 I.U.) with sucrose (30 mg) in 3 ml vials

		50°C				45°C				37°C			
	T=0	1W	2W	3W	5W	2W	4W	8W	10W	3W	5W	9W	12W
Batch 1	141	135	113	136	140	*135	*149	179	166	147	145	149	122
Batch 2	152	144	126	132	*146	135	167	160	162*	154	146	152	175

W = Weeks

* only one assay valid

temperature of 45°C or for 12 weeks at the temperature of 37°C. Again, no activity variation has been observed at all the test temperatures for both batches.

The stability forecast at room temperature, given in Tab. 6 and extrapolated from the accelerated stability data of Tab. 5 according to the Garret's method (Garret E.R., J. Pharm. Sci., 51:811, 1962) shows a degradation of about 35% and 80% after two years of storage at 4°C and 25°C respectively for the lactose containing formulations.

No degradation is foreseen at 4°C for the formulations with sucrose or sucrose plus glycin, whereas only a 6% decrease is foreseen for the sucrose containing formulations after two years at 25°C.

The stability has been studied of recombinant LH formulations (75 I.U.) with 50 mg sucrose (Composition a) and 50 mg lactose (Composition b).
The exact composition of recombinant LH formulations is given in Table 7.

The study of the accelerated stability of such formulations stored at 37°C, 45°C and 50°C, determined through the biological activity test measured in I.U. (Table 8) shows what has been already observed for the FSH formulations: the degradation of the sucrose containing preparations is extremely low, whereas the degradation of the lactose containing formulations is more evident.

The stability forecast at room temperature stability extrapolated from the accelerated stability data of Table 8 according to the Garret's method (Garret E.R., J.Pharm.Sci.,51:811, 1962) is given in Table 9.

A degradation is calculated of about 20% and 8% respectively for the lactose formulations stored for two

Tab. 6

Stability forecast of recombinant FSH formulations (150 I.U.) at room temperature

Composition	Excipient	Activity recovery (%)			
		4°C		25°C	
		1 year	2 years	1 year	2 years
a	Lactose	79.30%	62.89%	41.57%	17.28%
b	Sucrose	99.61%	99.22%	96.86%	93.82%
c	Sucrose + Glycin	no degradation		no degradation	

Tab. 7

Composition of recombinant LH formulations (75 I.U.) with sucrose 50 mg (Composition a) and lactose 50 mg (Composition b) in 3 ml vials

Composition	Excipient	Amount (mg)
a	Sucrose	47.75
	$NaH_2PO_4 \cdot H_2O$	0.052
	$Na_2HPO_4 \cdot 2H_2O$	0.825
b	Lactose	50.00
	$NaH_2PO_4 \cdot H_2O$	0.052
	$Na_2HPO_4 \cdot 2H_2O$	0.825

Tab. 8

Study of the accelerated stability of recombinant LH formulation (75 I.U.) in 3 ml vials

Excipient mg	T=0	50°C 1W	2W	5W
Sucrose 47,75	71 (58-86)	67* (34-121)	55 (42-73)	59 (47-76)
Lactose 50	77 (64-93)	57* (37-81)	34* (15-56)	40 (32-50)

Tab. 8 (Cont.)

Excipient mg	45°C 2W	5W	8W	12W	37°C 6W	9W	12W
Sucrose 47,75	65 (50-85)	-	59 (47-73)	57 (44-75)	67* (51-86)	70* (51-96)	72 (55-94)
Lactose 50	39 (29-52)	50* (33-79)	36* (20-57)	-	44* (32-60)	42* (31-56)	48 (38-62)

* Only one assay valid
(In brackets the confidential limits)
W = weeks

Tab. 9

Stability forecast of recombinant LH formulations (75 I.U.) at room temperature

Compositions	Excipient	Activity recovery % after 2 years	
		4° C	25° C
a	Sucrose	99.68%	90.65%
b	Lactose	80.56%	19.86%

years at 4°C and 25°C. The sucrose containing formulations remain unchanged for two years at 4°C and a decrease of only 9% is calculated for the same formulations after two years at 25°C.

A study has been also performed on urinary hCG formulations by using sucrose (formulation "a", 30 mg sucrose), lactose (formulation "b", 10 mg lactose) or mannitol (formulation "c", 20 mg mannitol) as stabilizers in 3 ml vials containing 500 I.U./vial hCG.

Tab. 10 gives the estimated values derived by the biological assay performed at different times for said hCG formulations stored at a temperature of 55°C.

Once again, sucrose is shown to be the most suited excipient in order to preserve hCG stability, i.e. an excipient which is much better than mannitol and better than lactose, even if, in this case, the stability difference for the three formulations is less strong with respect to the FSH or LH case.

EXAMPLES OF PHARMACEUTICAL MANUFACTURING

Materials: extra pure sucrose Ph Eur, BP, PH Nord, NF (Merck); lactose RPE ACS (Carlo Erba); glycin for analysis use (Merck), $NA_2HPO_4.2H_2O$ for analysis use (Merck), $NaH_2PO_4.H_2O$ RPE (Carlo Erba); 85% phosphoric acid RPE ACS (Carlo Erba); 0.1 M NaOH (Merck); water for injectables.

As containers, 3 or 5 ml glass vials have been used (type I borosilicate glass) with rubber fastener (Fradagrada Pettenati and Pharmagummi, butyl mixture) and aluminum ring.

Preparation of the sucrose containing recombinant FSH solution (for 1,200 vials containing each 150 I.U. FSH)

Sucrose (36 g) $Na_2HPO_4.2H_2O$ (1.33 g) and $NaH_2PO_4.H_2O$ (0.54 g) are dissolved into water for

Tab. 10

Study of stability at 55°C of hCG formulations (500 I.U.) with sucrose (a), lactose (b) and mannitol (c)

Composition	T=0	3W	6W
a	511 (390.1-670.2)	567 (407.0-788.7)	597 (452.2-789.4)
b	534 (396.7-719.6)	355 (293.7-430.2)	428 (330.2-55501)
c	449 (330.2-611.7)	332 (259.0-425.5)	244 (201.8-295.9)

W = weeks (Between brackets confidential 95% limits)

injectables (1,200 ml) in order to obtain the starting sucrose solution. The bulk of recombinant FSH (180,000 I.U.) is diluted with the solution so that an FSH solution is obtained at 200 I.U./ml.

The pH of the FSH solution and of the residual sucrose solution is adjusted at 7 by means of 0.1 M NaOH or H_3PO_4. The FSH containing solution is filtered through a Durapore 0.22 um sterile filter and brought to the final volume with the residual excipients solution filtered through the same Durapore filter.
During the process the solution temperature is kept between 4° and 8°C.

Preparation of the sucrose containing recombinant LH solution (for 1,200 vials each containing 75 I.U. LH)

Sucrose (57.3 g), $Na_2HPO_4.2H_2O$ (0.99 g) and $NaH_2PO_4.H_2O$ (0.62 g) are dissolved into water for injectables (600 ml) in order to obtain the starting sucrose solution. The recombinant LH bulk (90,000 I.U.) is diluted with the sucrose solution so that an LH solution is obtained at 300 I.U./ml.

The pH of the LH solution and of the residual sucrose solution is adjusted at 8 by means of 0.1 M NaOH or H_3PO_4. The LH containing solution is filtered through a 0.22 um Durapore sterile filter and brought to the final volume by means of the residual excipients solution filtered through the same Durapore filter. During the process the solution temperature is kept between 4° and 8°C.

The solutions containing different excipients have been prepared in a similar manner.

Filling up and lyophilisation

3 ml or 5 ml vials are filled up with 1 ml of FSH solution or 0.5 ml of LH solution, transferred to the

freeze-dryer and cooled at -45°C for 6 hrs. at least. The lyophilisation is started at the temperature of -45°C with a 0.07 vacuum. The heating is performed according to the following scheme: +20°C for 20 hrs., then +35°C until the end of the cycle.

On the reconstituted solution the usual quality controls have been performed.

Although the present invention has been illustrated by means of specific examples, it is understood that variations can be introduced without departing from the spirit and scope of the invention.

CLAIMS

1. A pharmaceutical composition comprising a solid intimate mixture of gonadotropin and a stabilizing amount of sucrose alone or in combination with other excipients.

2. A pharmaceutical composition according to Claim 1, wherein the solid intimate mixture is a lyophilisate.

3. A pharmaceutical composition according to Claims 1 and 2, wherein the gonadotropin is FSH, or LH or hCG.

4. A pharmaceutical composition according to any of Claims 1 to 3, wherein the gonadotropin is recombinant.

5. A pharmaceutical composition according to any of Claims 1 to 4, wherein the stabilizing agent is sucrose alone.

6. A pharmaceutical composition according to any of Clams 1 to 4, wherein the stabilizing agent is sucrose in combination with glycin.

7. A pharmaceutical composition according to any of Claims 1 to 6, containing 75 or 150 I.U. of FSH and 30 mg of sucrose.

8. A pharmaceutical composition according to any of Claims 1 to 6, containing 75 or 150 I.U. of LH and 47.75 mg of sucrose.

9. A process for preparing a pharmaceutical composition according to any of Claims 1 to 8, comprising the preparation of an aqueous solution of the

components, the distribution within containers and the drying or lyophilisation in the containers.

10. A process for preparing a pharmaceutical composition according to any of Claims 1 to 8, comprising the preparation of an aqueous solution of the components, the drying or lyophilisation of said solution and the distribution of the obtained solid mixture within containers.

11. A process according to Claims 9 and 10, wherein the pH of the solution is within the range 6.5 - 8.5.

12. A process according to Claim 11, wherein the pH of the solution is 7 for the FSH formulation and 8 for the LH formulation.

13. Forms of presentation of said pharmaceutical composition comprising the solid mixture according to any of Claims 1 to 8, hermetically closed in a sterile condition in a container suited for storage before use and reconstitution of the mixture in a solvent or a solution for injectables.

14. A solution comprising the solid mixture according to Claim 13, reconstituted in a solvent or a solution for injectables.

INTERNATIONAL SEARCH REPORT

International Application No PCT/IT 92/00165

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/38; A61K47/26

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	WO,A,8 810 270 (INSTITUTO DI RICERCA CESARE SERONO SPA) 29 December 1988	1-14
Y	see page 19 - page 21; example 3	1-14
X	EP,A,0 388 223 (APPLIED RESEARCH SYSTEMS ARS) 19 September 1990	1-5,7-14
Y	see column 7, line 14 - line 24; claim 1	6
X	EP,A,0 448 146 (AKZO N.V.) 25 September 1991 cited in the application	1-5,7-14
Y	see the whole document	6
Y	US,A,3 816 617 (BANIK) 11 June 1974 see column 3, line 41 - line 49	1-14

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° Special categories of cited documents : [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25 FEBRUARY 1993	08. 03. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	SITCH W.D.C.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	Section Ch, Week 9025, Derwent Publications Ltd., London, GB; Class B04, AN 90-188387 & JP,A,2 121 933 (KAGAKU SHIRYO KENKY) 9 May 1990 see abstract	1-14
Y	Section Ch, Week 8036, Derwent Publications Ltd., London, GB; Class B04, AN 80-62366C & DD,A,141 996 (WOLF I) 5 June 1980 see abstract	6
Y	WO,A,8 910 407 (GENETICS INSTITUTE,INC.) 2 November 1989 cited in the application see page 6 - page 9; examples 1,2	6
Y	WO,A,8 909 610 (CETUS CORPORATION) 19 October 1989 cited in the application see page 8, line 5 - page 10, line 6	6
A	WO,A,8 501 959 (INTEGRATED GENETICS,INC.) 9 May 1985 cited in the application see page 1, line 20 - page 3, line 22	
A	US,A,3 637 640 (HUBER) 25 January 1972 cited in the application see the whole document	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

IT 9200165
SA 68572

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 25/02/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8810270	29-12-88	AU-B-	615930	24-06-88
		AU-A-	1962088	19-01-89
		CN-A-	1030589	25-01-89
		EP-A-	0322438	05-07-89
		JP-T-	2500840	22-03-90
		US-A-	5128453	07-07-92
EP-A-0388223	19-09-90	US-A-	5087615	11-02-92
		CA-A-	2012170	17-09-90
		JP-A-	3246231	01-11-91
EP-A-0448146	25-09-91	AU-B-	631730	03-12-92
		AU-A-	7362391	26-09-91
		JP-A-	4217630	07-08-92
US-A-3816617	11-06-74	None		
WO-A-8910407	02-11-89	AU-B-	629549	08-10-92
		AU-A-	3538489	24-11-89
		EP-A-	0411033	06-02-91
		JP-T-	3504008	05-09-91
WO-A-8909610	19-10-89	AU-A-	3447389	03-11-89
		EP-A-	0423244	24-04-91
		JP-T-	3504721	17-10-91
WO-A-8501959	09-05-85	EP-A-	0160091	06-11-85
		EP-A-	0160699	13-11-85
		JP-T-	61500251	20-02-86
US-A-3637640	25-01-72	None		